UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY ANALYSIS OF SWEET POTATO (IPOMOEA BATATAS L.) GERMPLASM FROM MALAYSIA AND INDONESIA USING RAPD MARKERS

RAMISAH BTE MOHD SHAH

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By

RAMISAH BTE MOHD SHAH

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Institute of Bioscience Universiti Putra Malaysia

September 2001

UPM

Success

This success begins with our own will…
It's all in the state
of MIND.
Life battles are not always won.
By those who are stronger or faster,
Sooner or later
The person who wins
Is the person who thinks he can

*May **ALLAH** always*
Give the STRENGTH to all of us
and keep us in his BLESSING all over a year.
AMIN…

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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RAMISAH BTE MOHD SHAH

September 2001

Chairman: Associate Professor Dr. Mohd Said b. Saad

Faculty: Institute of Bioscience

Genetic variation among sweetpotato accession (*Ipomoea batatas* L.) from Malaysia and Indonesia has not been extensively examined with molecular markers. The objectives of this study are to use random amplified polymorphic DNA (RAPD) marker to determine the degree of polymorphism in the sweetpotato germplasm and to study the genetic diversity and relationships among sweetpotato accessions from two different regions, Malaysia and Indonesia.

A total of 92 accessions of sweetpotato germplasm from the two countries maintained at Universiti Putra Malaysia were characterized using five RAPD markers. Thirty-nine accessions were collected from seven different states of Malaysia and 53 accessions came from two different sub-regions of Indonesia (Irian Jaya and Java).

The results of this study indicated that the levels of polymorphism among all sweetpotato were extremely high. From five random primers

used (OPB 07, OPC 10, OPD 01, OPD 06 and OPG 14), 194 fragments were amplified, of which 192 (98.97%) were polymorphic. Only two fragments were monomorphic. The fragment size ranged from 117bp - 3240bp.

An NTSYS-pc computer program was further employed for data analysis using Jaccard's coefficient of similarity as a base for dendrogram construction via the UPGMA method. The Jaccard's similarity values ranged from 0.08 to 0.69 showing high levels of genetic variability among sweetpotato accessions. The cluster analysis separated Malaysian and Indonesian accessions into a different group with a number of additional clusters. Some of the Malaysian and Indonesian accessions were clustered based on their geographic source. The analysis indicates that very large genetic variation exists among sweetpotato accessions used in this study and the sweetpotato collection is a valuable as a genetic resource. This could be done by selecting cultivars from different groups delineated by cluster analysis for hybridization programs.

Genetic diversity analysis within the sweetpotato germplasm collection had provided useful information for managing this collection. RAPD appears to be useful for discerning variation within crop germplasm and to assess the genetic relationships among sweetpotato germplasm from Malaysia and Indonesia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS DIVERSITI GENETIK DI DALAM GERMPLASMA KELEDEK (*IPOMOEA BATATAS* L.) DARI MALAYSIA DAN INDONESIA MENGGUNAKAN PENANDA RAPD

Oleh

RAMISAH BTE MOHD SHAH

September 2001

Pengerusi: Profesor Madya Dr. Mohd Said bin Saad

Fakulti: Institut Biosains

Variasi genetik dalam germplasma tanaman keledek (*Ipomoea batatas* L.) yang berasal dari Malaysia dan Indonesia belum dikaji dengan mendalam menggunakan teknik penanda molekul. Objektif utama kajian ini adalah untuk menggunakan penanda RAPD bagi menilai darjah polimorfisma di dalam germplasma keledek dan mengkaji diversiti dan pertalian genetik di antara aksesi keledek dari dua kawasan yang berbeza, Malaysia dan Indonesia.

Sejumlah 92 aksesi germplasma keledek dari dua negara di pelihara di Universiti Putra Malaysia telah di cirikan dengan menggunakan lima penanda RAPD. 39 aksesi diperolehi dari tujuh negeri di Malaysia and 53 aksesi lagi dari dua sub-kawasan di Indonesia (Irian Jaya dan Jawa).

Keputusan daripada kajian ini menunjukan paras polimorfisma di antara tanaman keledek ini adalah sangat tinggi. Dari lima primer rawak yang

digunakan (OPB 07, OPC 10, OPD 01, OPD 06 dan OPG 14), 194 frakmen telah diamplifikasikan di mana 192 (98.97%) daripadanya adalah polimorfik. Hanya dua frakmen adalah monomorfik. Saiz frakmen adalah di antara 117bp – 3240bp.

Program komputer NTSYS-pc digunakan untuk mengalisis data menggunakan koeffisien persamaan Jaccard sebagai asas untuk membina dendrogram berdasarkan teknik UPGMA. Nilai anggaran persamaan adalah di antara 0.08 – 0.69 menunjukkan paras kepelbagaian genetik yang tinggi di antara aksesi keledek. Analisis kelompok yang dihasilkan telah mengasingkan aksesi Malaysia dan Indonesia dengan beberapa kelompok tambahan. Ada aksesi dari Malaysia dan Indonesia dikelompokkan berdasarkan kepada sumber geografi aksesi tersebut. Keputusan ini menunjukkan terdapat variasi genetik yang luas di antara aksesi keledek dan koleksi keledek ini berguna sebagai sumber genetik. Ianya dapat dilakukan dengan memilih kultivar dari kumpulan yang berbeza hasil daripada analisis kelompok di dalam program hibridisasi.

Analisis variasi di dalam koleksi germplasma keledek ini dapat memberikan pengetahuan yang berguna bagi tujuan pengurusan. RAPD didapati berguna untuk tujuan pengecaman variasi di dalam germplasma tanaman dan untuk menentukan pertalian genetik di antara germplasma keledek dari Malaysia dan Indonesia.

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I certify that an Examination Committee met on 10th September 2001 to conduct the final examination of Ramisah bte Mohd Shah on her Master of Science thesis entitled "Genetic Diversity Analysis of Sweetpotato (*Ipomoea batatas* L) Germplasm from Malaysia and Indonesia using RAPD Markers" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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10 JAN 2002

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

RAMISAH BTE MOHD SHAH

Date: 16 NOV 2001

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LIST OF ABBREVIATIONS AND GLOSSARY

A260nm	: Absorbance at 260nm
A280nm	: Absorbance at 280nm
AFLP	: Amplified fragment length polymorphism
AP-PCR	: Arbitrary primed polymerase chair reaction
bp	: base pair
CIP	: International Potato Center
DAF	: DNA amplification fingerprinting
dATP	: deoxyadenine-5'-triphosphate
dCTP	: deoxycytidine-5'-triphosphate
dGTP	: deoxyguanocine-5'-triphosphate
DNA	: Deoxyribonucleic acid
dNTP	: deoxynucleotide-5'-triphosphate
dTTP	: deoxythymidine-5'-triphosphate
EDTA	: Ethylenediamine tetra-acetic acid
FAO	: Food and Agriculture Organization
IPGRI	: International Plant Genetic Resource Institute
Kb	: Kilobase
M	: Molar
$MgCl_2$	: Magnesium chloride
Min	: minute (s)
mM	: milimolar
ηg	: nanogram
NaCl	: Sodium chloride

OD	:	Optical density
PCR	:	Polymerase chain reaction
RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
rpm	:	revolution per minute
SSR	:	Simple sequence repeat
Taq	:	*Thermus aquaticus*
TBE	:	Tris-borate EDTA
TE	:	Tris-EDTA
µg	:	microgram
µl	:	microliter
UV	:	Ultraviolet

GLOSSARY

Amplification	The production of many DNA copies from one master region of DNA.
Anneal	The spontaneous pairing of complementary DNA or RNA sequences by hydrogen bonding to form a double-stranded polynucleotide.
Arbitrary primer	A short oligonucleotide primer used in certain PCR methods to initiate DNA synthesis at random locations on the target DNA.
Base	The chemical unit which characterises a nucleotide. In DNA the bases found are adenine, guanine, thymine and cytosine.
Base pair	Two nucleotide bases on different strands of a nucleic acid molecule that are held together by hydrogen bonds. Bases can pair in only one way – adenine with thymine and guanine with cytosine in DNA.
Cross-pollination	The fertilization of one plant with pollen from another. This outcrossing ends to enhance genetic diversity in plant populations because more diverse genetic mixtures occur.
Cultivar	A contraction of cultivated variety.
Dendrogram	A graphical representation of the results of a clustering procedure in which the vertical axis consists of the objects or individuals and the horizontal axis represents the number of clusters formed at each step of the clustering procedure.
DNA	Deoxyribonucleic acid – genetic material found in all living organisms. Every characteristic of every living organism can be traced to the code of its DNA.
Electrophoresis	A technique for separating molecules in a matrix (such as agarose or starch gels) according to their electrical charge and size.
Enzyme	A specialized protein catalyses biochemical reactions.
Gene	The chemical units of heredity that, when expressed, determine an organism's traits.

Genetic diversity	The range of genetic differences among individuals or groups of organisms.
Genetic resources	Germplasm containing potentially useful characteristics of plants, animals and other organisms.
Genotype	The genetic constitution of an individual or group that may be either expressed or unexpressed, depending on the environmental effects of a given location.
Germplasm	The collection hereditary materials within a species.
Heterosis	The intensified expression of desirable genetic traits that makes a hybrid superior to its parents.
Hybridization	A procedures that involves the deliberate act of applying male pollen onto the female stigma to effect fertilization.
Hybrid vigor	The intensified expression of desirable genetic traits that makes a hybrids superior to its parents.
Isozymes	Variations of an enzyme
Loci	Plural of locus.
Locus	A specific site on a chromosome, usually of a gene or other marker.
Marker	An identifiable physical location on a chromosome whose inheritance can be monitored.
Monomorphic	The situations in which all the individuals in a population are the same genetic type or have the same allele.
Mutation	Alterations in the genetic code due to environmental influences or errors in replication.
Natural selection	Nature's selection of organism that adapt best to environmental and/or hereditary changes.
Polymorphism	A detectable difference at a particular marker occurring among individuals.

Primer	A short DNA fragment annealed to a single-stranded DNA, to which further nucleotides can be added by DNA polymerase.
Selection	To discriminate deliberately among individuals in the number of offspring contributed to the next generation.
Species	In taxonomy, the next step below genus. Individuals within a species often look alike and can interbreed.

CHAPTER 1

INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is an important food crop which is grown in a wide climatic conditions located between 15ºS and 45ºN (MacKay, 1989). It currently ranks seventh among the most important food crops after rice, wheat, maize, potato, barley and cassava (FAO, 1997). World sweetpotato production exceeds 140 million tons in an area of about 9.4 million ha (FAO, 2001).

Sweetpotato has been regarded as the 'potato' of the warm tropics due to its ability to grow under high temperatures and low inputs of water and fertilizer (Bohac *et al.,* 1995). Sweetpotato has and will play an important role in solving global issues associating food, natural resources and the environment of the 21st century (Kozai *et al.,* 1997).

Sweetpotato exhibits great diversity in morphological and phenotypic traits, such as growth habits, leaf shape and storage root flesh and skin color (Woolfe, 1992; Saad, 1993). There are thousands of different sweetpotato genotypes cultivated around the world. In Papua New Guinea, it is estimated that there are about 5000 cultivars of sweetpotatoes. The island of New Guinea has been considered as the secondary center of diversity for sweetpotato because of its range of

isolated ecological niches and the large number of cultivars found within a small area (Zhang *et al.*, 1998).

Saad (1993) reported that some Malaysian farmers grow more than 10 cultivars at one time. Most sweetpotato farmers grow their own cultivars. Many of these cultivars were not selected, but some farmers selected their own cultivars from several varieties obtained from other farmers or their friends. Nevertheless, some advanced farmers have brought in varieties from other countries such as China and Indonesia (Saad and Anang, 1994).

Many of these cultivars have arisen through systematic breeding efforts, but an appreciable number of them haves also arisen through natural hybridization and mutations. Sweetpotato is hexaploid and cross-pollinated. Continuous outcrossing between different genotypes leads to formation of many segregants. The crop's outcrossing nature, combined with vegetative propagation capabilities has created a vast number of cultivated genotypes around the world.

Sweetpotato germplasm collection, characterization and conservation are important prerequisites for the utilization in crop improvement programmes. To facilitate efficient germplasm collection and management practices, there is a continual need for a greater understanding of the extent of genetic variation within the germplasm

collections and the nature of genetic relationships among the accessions.

Genetic variation within and between populations of crop is a major interest to plant breeders and populations geneticists. Knowledge of the distribution of genetic diversity is essential for rational germplasm conservation. Information on genetic identity and relationships of genotypes is crucial to the development of source materials or core collection (Frankel, 1984). A core collection is essential for rationalizing the management and enhancing the utilization of the genetic diversity available in the entire sweetpotato germplasm collection. A core collection contains a subset of accessions from entire collection that captures most of the available genetic diversity of the species (Brown, 1989).

Genetic variation assessments of agricultural species traditionally are based on differences in morphological and agronomic characteristics. These types of data are often influenced by environmental factors. In recent years a variety of molecular techniques have been developed for measuring genetic variability in plant genetic collections.

Molecular markers can afford many benefits for identifying variation and estimating biological diversity (Virk *et al.*, 1995). These techniques allow researchers to identify accessions at the taxonomic level, assess the

relative diversity within and among species and locate diverse accessions for breeding purpose.

Random amplified polymorphic DNA (RAPD) has been well established in the past few years as a cost-effective means of assessing genetic variation at the DNA sequence level without requiring a prior knowledge of species DNA sequences (Williams *et al.,* 1990; Hadrys *et al.*, 1992; and Huff *et al.*, 1993). These techniques are widely accepted and have been used successfully for different purposes, such as to investigate the genetic relationships between different cultivars (Moeller and Schaal, 1999), to construct genetic maps (Eujayl *et al.,* 1998), to identify molecular markers linked to genes of interest (Nair *et al.*, 1996) and to detect genetic diversity (Wachira et *al.*, 1995).

The objective of this study were i) to determine the degree of RAPD polymorphism in the sweetpotato germplasm and ii) to study the genetic relationship between the Malaysian and Indonesian sweetpotato accessions.